Remarks

Applicants thank the Examiner for pointing out the typographical error in Claim 16. This amendment/response corrects this typographical error and a few other obvious typographical errors in the previously submitted amendment. Applicants believe the resubmitted amendment complies with the requirements of revised 37 CFR 1.121. The arguments in the response are the same as in the previously submitted response, but are being resubmitted, along with the corrected claims, for the convenience of the Examiner.

Claims 1, 4-14, 16-24 and 36 are pending in the present application. Claims 3 and 25-35 have been withdrawn to limit the pending claims to cover only the elected subject matter of Group I. Applicants reserve the right to file a divisional case including these claims at a later date. Claim 1 has been amended to clarify the claim language, to correct for insufficient antecedent basis and to specify that the PCR products are approximately 10 kb to approximately 15 kb. Support for this amendment can be found on page 2, lines 11-12 of the specification and p. 4, lines 16-18. Claim 2 was cancelled to expedite prosecution of the remaining claims. Claims 5 and 6 were amended to include limitations that the PCR products are approximately 10 kb to approximately 15 kb and that they encompass the entire chromosome. Support for these amendments can be found on page 2, lines 11-13 of the specification. Claim 7 was cancelled because it was redundant in view of amended Claim 5. Claims 10 – 14 and 16-21 have been amended to have proper antecedent basis for amended Claim 5 and Claim 1. Claims 16-21 were also amended to clarify that "bacteria" refers to "wild-type" bacteria and to remove dependency from cancelled Claim 2. Support for this amended is found on p. 2, lines 10-29. Claim 15 was cancelled because it was redundant in view of amended Claim 6. Claims 22-24 were amended to have proper antecedent basis in view of the amendment to claim 6. Claim 36 was amended to incorporate the amendments made in Claims 1, 5 and 6. Examiner is respectfully requested to reconsider the application.

The present invention allows the identification of mutations in genes and gene products that confer resistance to a chemical. This can be done without any prior information about the location of the mutation in the chromosome or the mechanism of action of the chemical.

Claim Rejections under 35 USC §112

The numbering used below refers to the numbering used in the Examiner's office action of August 9, 2002.

Claim Rejections under 35 USC §112, first paragraph

(2) The Examiner has rejected Claim 1, 4-24 and 36 under 35 USC §112, first paragraph because the specification "does not reasonably provide enablement . . . commensurate in scope with these claims." The Examiner cites to factors from Ex parte Forman, 230 USPQ 546, 547 (Bd. Appl 1986) the analysis from which the Examiner concludes that undue experimentation would be required to practice the invention. Applicants will address each of these factors in turn to explain why the Applicants believe the invention is fully enabled.

(a) The quantity of experimentation necessary.

The Applicants have amended the claims to point out that the organism used is a bacterial strain for which the wild-type chromosomal sequence is known at the time the method is carried out. One of skill in the art would be able to readily determine which bacterial strains have a chromosomal sequence available. Methods of primer selection are described in the specification (page 20, line 15 to page 25, line 20. Page 23, line 26 through page 24, line 26.) Methods of transformation, PCR, and isolation of resistant compounds are well known in the art. Applying this knowledge with the disclosure of the present application, one of skill in the art would need little, if any, experimentation to be able to perform the methods of the present invention. Applicants acknowledge that the methods of the present invention are multi-step and it may require a considerable amount of time to reach the final step in which mutations are identified. However, the technology required for these steps is well within the purview of one of skill in the art and would be considered routine and therefore does not amount to undue experimentation.

(b) The amount of direction presented

The Examiner states that the "specification provides guidance only for in the gyrA and quinolone resistance determining region." Applicants respectfully disagree. The specification provides specific directions as to how one would mutate the entire

chromosome as well as to do all the required steps of the methods. See in particular the Materials and Methods section from page 20, line 15 to page 25, line 20. Page 23, line 26 through page 24, line 26, and the disclosure on page 18, line 15 through page 20 line 14. These sections specifically describe using PCR on large regions of the chromosome or the entire chromosome to identify both PCR generated mutations as well as mutation that have been generated by other means. These sections discuss selection of primers, and generation of the mutated fragments. Page 24, line 27- page 25, line 20 describes transformation of sensitive bacteria, selection of resistant strains, and subsequent steps to arrive at the mutation which conferred the resistance phenotype.

(c) The presence or absence of working examples

The Examiner correctly points out that Applicants have several working examples. However, the Examiner states that "working examples are presented only for . . . " The use of "only" by the Examiner seems to imply that these working examples are insufficient. Applicants submit that in the context of the full specification these working examples are adequate to enable the full scope of the invention. Applicants would like to point out that each of these factors is a part of the enablement analysis and that there is no requirement for particular types or numbers of working examples.

(d) The nature of the invention

The claims have been amended to clarify that the invention is directed to a process for identifying and characterizing mutations that confer resistance to a compound in bacteria for which the relevant chromosomal sequence is known at the time of performance of the process. Thus, the Examiner's characterization of the invention is not correct.

(e) The state of the prior art

The prior art is relevant to the present invention in that the technologies utilized by the present invention are well known in the art. One of skill in the art would be familiar with high and low fidelity PCR, with primer design and with selection of bacterial strains that exhibit a particular phenotype.

(f) The relative skill of those in the art

As noted by the Examiner "the level of skill in the art of molecular biology is high." Thus, the specification does not have to provide every single detail for carrying out the

present invention. It is the disclosure of the specification combined with the knowledge of one of skill in the art that must enable the use of the claimed invention.

(g) The predictability of the art

The Examiner states that "the results of experiments involving any organism randomly mutated and transformed, wherein a mutation confers bacteria resistant to any compound, is not predictable." Applicants submit that the specification of the present application provides enablement sufficient to address any unpredictability of mutation and transformation. By using larger PCR products Applicants have shown that following random mutagenesis, mutations that confer resistance can be identified.

(h) The breadth of the claims

The Examiner states, "the claims are broadly drawn, reciting any possible mutation in any organism leading to bacteria resistant to any compound." Applicants point out that the pending claims are to a process which has been amended to clarify that at the time the method is carried out, the wild-type sequence of the bacterial chromosome is known. The claim is not drawn "to any possible mutation in any organism" it is drawn to a **process** for identifying mutants." As such the claims are not overly broad.

Given the evaluation of the factors above and the teachings in the specification and in the prior art, Applicants submit that one of skill in the art would be able to perform all the step of each of the methods without undue experimentation.

(3) The Examiner has also rejected Claims 2 and 16-21 under 35 USC §112, first paragraph for particular language "help to understand the mechanism of action of quinolones, and other type IV topoisomerase inhibitors" found in Claim 2 as originally filed. Claim 2 has been cancelled to expedite prosecution of the remaining claims and Claims 16-21 have been amended to remove their dependency from Claim 2. Claims 16-21 continue to be dependent from Claim 1. Applicants submit that the rejection under 35 USC §112, first paragraph, is moot with respect to cancelled Claim 2 and is no longer applicable to Claims16-21.

Claim Rejections under 35 USC §112, second paragraph

(5) The Examiner has rejected Claims 1, 4, 8 and 16-21 under 35 USC §112, second paragraph. The Examiner objects to the language "12 PCR products corresponding to 100 kb of the chromosome." Although Applicants believe that in the context of the specification that the meaning of this phrase is clear, Claim 1 has been amended to substitute the following language "transforming pools of the PCR products from step (a) corresponding to about 100 kb of the chromosome" to indicate clearly that the sum total of the pooled PCR products is about 100 kb for a given transformation. Support for this amendment can be found on p. 4, lines 16-18, of the specification.

The Examiner requests correction and clarification of the use of the language "wild type background". This language would be clear to one of skill in the art in the context of the specification and in the context of the present invention. To make the claim language more clear, Claim 1 has been amended to read "wild-type strain" instead of "wild-type background". In the context of any of the claims, "wild-type strain" means a bacterial strain having the chromosomal sequence prior to mutagenesis and selection for resistance. Thus, a "wild-type strain" is sensitive to the compound used for selection. Applicants believe that this language is clear in the context of the application. Support for this amendment is on p. 2, lines 10-29.

(6) The Examiner points out that in original Claim 1 the limitation "compound" in step c) had insufficient antecedent basis. Claim 1 has been amended and "compound" in step c) now has sufficient antecedent basis.

(7) The Examiner has rejected Claims 2 and 16-21 under 35 USC §112, second paragraph stating that step "e" of Claim 2 is "a confusing step". Claim 2 has been cancelled to expedite prosecution of the remaining claims and Claims 16-21 have been amended to remove their dependency from Claim 2. Claims 16-21 continue to be dependent from Claim 1. Applicants submit, therefore that this rejection is moot with respect to cancelled Claim 2 and is no longer applicable to Claims 16-21.

(8) The Examiner has rejected Claims 2 and 16-21 under 35 USC §112, second paragraph for reciting the phrase "wild type background. Claim 2 has been cancelled to expedite prosecution of the remaining claims and Claims 16-21 have been amended to remove their dependency from Claim 2. Claims 16-21 continue to be dependent from

Claim 1. Applicants submit that this rejection is moot with respect to cancelled Claim 2 and is no longer applicable to Claims 16-21.

(9) The Examiner has rejected Claims 6 and 9, 15 and 22-24 under 35 USC §112, second paragraph because Claim 6 recites "generating DNA fragments by PCR amplification of the bacterial chromosome corresponding to regions of the bacterial chromosome which may contain a mutation." Claim 6 has been amended to more clearly point out that the PCR amplification to generate fragments is carried out on a strain of bacteria that have a mutation that confers resistance to a compound. Claim 6 has been further amended to recite that the DNA fragments generated are overlapping PCR products of approximately 10 kb to approximately 15 kb that encompass the complete chromosome from a strain of bacterial which demonstrates resistance to a compound. Applicants submit that amended Claim 6 clearly points out what is claimed and therefore is no longer subject to the Examiner's rejection. Likewise, Claims 9, 15 and 22-24, which are dependent from Claim 6, are no longer subject to the Examiner's rejection.

(10) The Examiner has rejected Claims 16-21 under 35 USC § 112, second paragraph for insufficient antecedent basis for the limitation "the antibacterial compound". Claim 16-21 have been amended to replace "the antibacterial compound" with "the compound" which has proper antecedent basis in Claim 1. Applicants submit that amended claims 16-21 are in compliance with 35 USC §112, second paragraph.

For the reasons above, Applicants submit that all claims are in compliance with the requirements of 35 USC §112, first and second paragraphs, and therefore request that the Examiner's rejections under these sections be withdrawn.

Claim Rejections under 35 USC §103

(12) The Examiner has rejected Claims 5, 10-11 and 36 as being unpatentable over Kok et al. (Journal of Bacteriology, July 1997, pp 4270-4276, Vol. 179, No.13) in view of Belland et al. (Molecular Microbiology, 1994, Vol. 14, No. 2, pp 371-382). The Examiner states that "Kok teaches a method to identify mutations in a gene altering the natural function of a protein encoded by said gene." The Examiner further states that "Mutations were mapped and the sequences analyzed" and that Kok "noted that analysis of a wide range of chromosomal point mutations should be applicable to other genes in

natural transformations systems and to study the structure-function relationship of proteins." The Examiner points out that "Kok does not teach that an antibacterial compound is used for the selection . . . or that the compound is an inhibitor of type II topoisomerases . . . or that the antibiotic compound is ciprofloxacin." The Examiner cites Belland as teaching "that the antibacterial compound used for selection of transformed bacteria is the fluoroquinolone ciprofloxacin.

To establish a *prima facie* case of obviousness under 35 USC §103(a), three basic criteria must be met: 1) The prior art reference must teach or suggest all the claim limitations; 2) There must be some suggestion or motivation to modify the reference or to combine reference teachings; and 3) There must be a reasonable expectation of success. Also, the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. MPEP 2142

The present invention as claimed in Claim 5 and dependent claims 10, 11 and 36 involves random mutagenesis of the complete chromosome using PCR to generate large DNA fragments of approximately 10 kb to approximately 15 kb. The present invention allows for the identification of mutations associated with resistance to a compound that are anywhere on the chromosome. By applying the methods of the present invention, even for compounds for which the mechanism of action is unknown, the user is able to identify genes that, when mutated, result in a decreased susceptibility to a compound. This allows one who is skilled in the art to predict the mechanism of action of any compound with no prior information concerning the molecular target of the compound. Before the disclosure of this invention it had not been shown that a mutation that conferred resistance to a compound could be systematically identified using PCR without prior knowledge of the region with which the compound interacted.

Kok et al. describe a PCR-based technique for targeted random mutagenesis of selected genes in a bacterial chromosome (Abstract, line 1, emphasis added and p 4275, paragraph 4 of the Discussion section) in order to gain insight into how structure influences the function of the protein encoded by the selected gene. Specifically Kok describes mutagenesis of *Acinetobacter pobR* which encodes the transcriptional activator

of *pob*A. Using a 1434 bp region containing *pobR* as a template, Kok discloses that PCR-mediated mutagenesis can generate mutations which eliminate or reduce the activity of PobR and thereby cause a decreased transcription of *pobA*. The level of expression of PobA can be conveniently measured because PobA expression results in the conversion of 4-hydroxybenzoate to a toxic metabolite. Reduction in the activity of PobR can be by loss-of-function mutations in *pobR* or promoter mutations in the *pobAR* intergenic region. Kok discloses that the transformation frequency for a mutant PCR product is 1 in 10^3 (Figure 2). Also presented is data that stop-codon revertants can be achieved via PCR-mediated mutagenesis however the frequency of the codon-specific mutagenesis is approximately 3 orders of magnitude lower than for the transformation of mutant PCR products (p 4274 1st paragraph). There is no analysis of whether compound-target interactions can be identified via PCR mutagenesis. The studies in Kok are focused on identifying mutations which cause a loss-of function of a known gene.

Belland, et al. describes the isolation and characterization of two *gyrA*-like genes from *N. gonorrhoeae*. Belland tentatively identifies these genes as the *N. gonorrhoeae gyrA* and *parC* homologues based on homology to these genes in *E.coli* and other proteobacteria. Because mutations in *gyrA* have been shown to play a role in fluoroquinolone resistance, Belland et al examined fluoroquinolone resistance in *N. gonorrhoeae* using mutants generated by serial passaging of *N. gonorrhoeae* on inhibitory concentrations of ciprofloxacin (spontaneous mutations). Belland et al analyzed the resistant mutants by amplifying short regions (<180bp) of *gyrA* and *parC* using PCR and then sequencing these regions to identify the particular sequence difference(s) in the mutation (results, table 3). Belland notes that mutations giving rise to ciprofloxacin resistance that occur outside the region analyzed may influence the level of ciprofloxacin resistance but that these mutation would not be detected by the procedures used in Belland (p377, 2nd column, lines 44-48). Belland does not disclose any method by which those mutations could be identified or characterized.

To ensure that the observed mutations are responsible for resistance the authors, in Belland, use chromosomal DNA from mutant strains as donor DNA and then determine the DNA sequence of the Quinolone-resistance determining region (QRDR) of

resistant transformants. The authors note that transformation frequencies for these studies are 1 in 2 x 10^3 for a particular mutant, FA19, (p. 377 line 8-10) and approximately 1 in 2 x 10^4 for another mutant, MS11, (p. 377 line 18-21). PCR products generated from mutant templates were used to ensure that the only mutation responsible for resistance in those mutant strains was located in *gyrA*. The transformation frequencies for this event were "at a frequency much lower than found with chromosomal DNA" (p. 377 lined 24-29) suggesting Neisseria can be transformed with PCR products but at a much lower frequency when compared to chromosomal DNA transformation.

The prior art does not teach or suggest all the claim limitations of the present invention.

Neither Kok alone, nor in combination with Belland, teaches or suggests to one of skill in the art that low fidelity PCR mutagenesis could be used over the entire chromosome to locate mutations responsible for resistance to a chemical compound. The present invention, as claimed in amended Claim 5 and dependent claims 10, 11 and 36, involves random mutagenesis of the complete chromosome using PCR to generate large DNA fragments of approximately 10 kb to approximately 15 kb and allows for the identification, anywhere on the chromosome, of mutations which conferring resistance to a compound.

Kok does not teach or suggest that it would be desirable or possible to randomly mutagenize the entire chromosome of a bacterium. Nor does Kok teach or suggest the use of PCR products of approximately 10 kb to approximately 15kb. Kok et al. describe a PCR-based technique for targeted random mutagenesis of selected genes in a bacterial chromosome. Kok discloses only mutagenesis of a known 1434 bp region around *pobR*. Kok does state that "[t]he technique is of general interest because it may be widely applicable in mutagenesis of other chromosomal genes." However, this would not suggest to one of skill in the art that PCR-mediated mutagenesis could used at the chromosomal scale and does not teach or suggest the use of approximately 10 kb to approximately 15 kb DNA fragments to do so.

Nothing disclosed in Belland rectifies the deficiencies of Kok. As discussed above, Belland discloses using chemical (ciprocloxacin) induced mutagenesis to generate

resistant strains and then confines the analysis of those mutants to short regions of the gyrA and parC genes which had previously been implicated in quinolone resistance. Belland does not disclose the use of PCR to generate mutants. Belland uses PCR amplification of short stretches of DNA (<180 bp) to sequence regions of *gyrA* and *par C* to identify mutations. Belland notes that mutations giving rise to ciprofloxacin resistance that occur outside the region analyzed may influence the level of ciprofloxacin resistance but that these mutation would not be detected by the procedures used in Belland (p377, 2nd column, lines 44-48). Belland does not disclose any method by which those mutations could be identified or characterized. Nothing in Belland teaches or suggests that PCR mutagenesis could be applied to a complete chromosome to generate mutants resistant to a compound or that overlapping PCR products of approximately10 kb to approximately 15 kb be used.

Thus, there is no motivation in Belland to modify the method of Kok to encompass the complete chromosome or to use overlapping PCR products of approximately 10 kb to approximately 15 kb. Even if the references are combined and the PCR mutagenesis method in Kok were applied to identify mutations which confer resistance to a compound such as ciprofloxacin, neither reference teaches or discloses a way in which to identify mutations associated with resistance to a compound that are anywhere on the chromosome. Both references deal with selecting for mutations associated with a phenotype for which the region/gene responsible for such phenotype is known. In contrast, with the methods of the present invention the user is able to identify genes that when mutated result in a decreased susceptibility to a compound, even for compounds for which the mechanism of action is unknown. This allows one who is skilled in the art to predict the mechanism of action of any compound with no prior information concerning the molecular target of the compound. Before the disclosure of this invention it had not been shown that a mutation which conferred resistance to a compound could be systematically identified using PCR without prior knowledge of the region with which the compound interacted.

There would not have been a reasonable expectation of success

While it may have been obvious to try low fidelity PCR mutagenesis to identify mutations that confer resistance to a compound, there would not have been a reasonable expectation of success that such a method would work. This is because for the frequencies of transformation expected, one of skill in the art would not have had a reasonable expectation that that one could search the entire chromosome using PCR and identify one or more mutations conferring resistance to a compound. In other words, before the disclosure of the present invention, it would not have been obvious that frequencies of transformation would be high enough to allow for selection of compound resistant mutants where the region or gene containing the mutation was unknown. Kok and Belland do not suggest to one of skill in the art that such a method would succeed. To the contrary, Kok and Belland disclose frequencies of transformation that support the unobviousness of the instant invention.

Kok discloses that the transformation frequency for a mutant PCR product is 1 in 10^3 (Fig 2). When Kok examines codon-specific phenotypes the frequencies decrease dramatically to approximately 1 in 10^6 (p 4274, lines 13-14, column 1). This change in frequency is a direct result of the number of mutations within a given gene that can result in the necessary phenotype. Because antimicrobial compounds generally target enzymes essential for the viability of a cell, it is impossible for loss of function mutations to result in resistance to a compound. Thus the frequency of identifying such mutants would be expected to be significantly lower and therefore to ensure success it is necessary to ensure that all codons across the complete chromosome are adequately mutagenized. One of skill in the art would recognize that the frequency that Kok observes for the "stop-codon" revertant studies should be a benchmark to evaluate effective mutagenesis with relation to generation of compound resistant mutants. Based on Kok et al's frequencies it is predicted that identifying a mutation in a particular codon will occur approximately 10^3 times less frequently than the transformation frequency observed for a mutant template. Belland does not specifically quantitate the transformation frequency of a PCR product containing the *gyrA* mutant alleles but does state that PCR product transformation was "at a frequency much lower than found with chromosomal DNA" (1 in 2×10^3 for FA1090, 1 in 10^4 for MS11). If one conservatively assumes that the transformation frequency of a

PCR mutated fragment is 1 in 10^4 (10-fold lower then chromosomal DNA – based on the disclosure of Belland) only 1 cell in 10^7 would be expected to contain a particular mutation. (based on the three orders of magnitude difference, in Kok, between the frequency of a null mutation and the frequency of mutation of a particular codon.) Such a frequency is very low and due to the practical constraints of screening a large number of transformants, one of skill in the art would not have concluded that such a method would be successful for identifying a mutation conferring a resistance phenotype.

Applicants assert that the Examiner is using hindsight construction, using Applicants' disclosure, to come to the conclusion that Claims 5 and 10-11 are obvious. Applicants assert that the data presented in Kok et al and Belland et al. would not have suggested to one of skill in the art, at the time the instant application was filed, that there was a reasonable expectation for these experiments to work to identify compound resistant mutants where the region or gene containing the mutation was unknown; thus requiring that the entire chromosome be analyzed to account for all possible mutations. Within the disclosure of Belland it is noted that mutations giving rise to ciprofloxacin resistance that occur outside the region analyzed may influence the level of ciprofloxacin resistance but that these mutation would not be detected by the procedures used (Belland, p377, 2nd column, lines 44-48). Kok and Belland disclose analysis of small regions of 1434 bp and <180 bp respectively. It is Applicants' disclosure that shows data which supports a method of mutating a complete chromosome by PCR, selecting compound resistant mutants and analyzing the complete chromosome to locate the mutation. Figure 3 of the present application demonstrates that the transformation efficiency of an approximately 9 kb PCR product from a wild-type template in *N. gonorrhoeae* can be achieved at a frequency of 1 in 10^2 CFUs. This result provides evidence that PCR-mediated mutagenesis using a large PCR product is an efficient means to identify ciprofloxacin-resistant alleles. With the data from Applicants' Figure 3, one of skill in the art would recognize that using larger sized fragments, such as used for Figure 3, would allow mutagenesis and analysis of a complete chromosome. It is only with such data that one of skill in the art would have a reasonable expectation of success that PCR-mediated mutagenesis of an entire chromosome and identification of the resulting resistant mutants would be feasible.

(13) The Examiner has rejected Claim 12 as being unpatentable under 35 USC §103(a), over Kok et al. (Journal of Bacteriology, July 1997, pp 4270-4276, Vol. 179, No.13) in view of Belland et al. (Molecular Microbiology, 1994, Vol. 14, No. 2, pp 371-382) as applied to Claim 5, and further in view of Pruna (US Pat No. 5,532,239). The Examiner states that it "would have been obvious to combine Kok and Belland and Pruna, as clinafloxacin is a well known antibiotic, and research on it would have been useful in determining which bacteria are likely to form a resistance to it, and what mutations cause a resistance to it."

Pruna discloses Fluoroquinolones for the treatment of nephrotic syndromes and discloses that clinafloxacin is, along with fourteen other compounds, especially advantageous. Pruna does not have any disclosure concerning the mechanism of action of clinafloxacin or provide any motivation to look for mutations that would cause resistance to clinafloxacin or any other compound.

Kok and Belland, as discussed above, neither alone or in combination teach or suggest all the elements of the claimed invention or provide a reasonable expectation of success for the present invention. The arguments apply to amended Claim 12 as well because it is dependent from Claim 5. Pruna does nothing to rectify the deficiencies of Kok and Belland. Even if the three references combined did suggest that it would be useful to study clinafloxacin, Pruna does not add anything that would change the above analysis for Kok and Belland. Pruna does not disclose PCR-mediated mutagenesis using approximately 10 to approximately 15 kb fragments encompassing a complete chromosome. And in no way does Pruna increase the expectation of success that such a method would succeed even where the compound is clinafloxacin.

(14) The Examiner has rejected Claims 13 and 14 under 35 USC §103(a), as being unpatentable over Kok et al. (Journal of Bacteriology, July 1997, pp 4270-4276, Vol. 179, No.13) in view of Belland et al. (Molecular Microbiology, 1994, Vol. 14, No. 2, pp 371-382) as applied above, and further in view of Ibrahim (US Pat No. 5,145,667).

Ibrahim discloses oral hygiene compositions that are useful in the inhibition of dental calculus and further discloses addition of an anti-bacterial compound to the composition to provide anti-plaque activity. Disclosed as suitable anti-bacterial agents are diphenyl ethers and specifically triclosan. Ibrahim has no disclosure relating to a method of mutagenesis or of identifying mutations that confer resistance to a compound.

Applicants do not contest that DHDPE and Triclosan are known antibiotics. The disclosure of Ibrahim, even if it does teach DHDPE and Triclosan, does nothing to make up for the deficiencies of Kok and Belland. As discussed in detail above, Claim 5 is not obvious and therefore claims dependent from it are non-obvious as well. Ibrahim does not disclose PCR-mediated mutagenesis using approximately 10 to approximately 15 kb fragments encompassing a complete chromosome. And in no way does Ibrahim increase the expectation of success that such a method would succeed even where the compound is triclosan or DHDPE.

(15) The Examiner has rejected Claims 6, 9 and 24 under 35 USC §103(a), as being unpatentable over Belland et al. (Molecular Microbiology, 1994, Vol. 14, No. 2, pp 371-382) in view of Jones et al. (Biotechniques, Vol. 10, No. 1, 1991, pages 62-66).

Jones et al describe a system for recombination that allows for recombination of a linear PCR product with itself to generate a functional plasmid that has incorporated a desired mutation. By relying on the ability of E.coli to recombine homologous ends the method of Jones avoids the need to form staggered ends *in vitro*. Jones et al. do not discuss bacterial chromosomes at all. There is no disclosure in Jones relating to a method for identifying a mutant in a bacterial chromosome.

The discussion above of Claim 5 and Belland apply here as well. Belland does not disclose or suggest all the elements of the Claim 6 (or of dependent claims 9 and 24). Belland discloses using chemical (ciprocloxacin) induced mutagenesis to generate resistant strains and then confines the analysis of those mutants to short regions of the gyrA and parC genes which had previously been implicated in quinolone resistance. Belland does not disclose the use of PCR to generate mutants. Belland uses PCR amplification of short stretches of DNA (<180 bp) to sequence regions of *gyrA* and *parC*

to identify mutations. Belland notes that mutations giving rise to ciprofloxacin resistance that occur outside the region analyzed may influence the level of ciprofloxacin resistance but that these mutation would not be detected by the procedures used in Belland (p377, 2nd column, lines 44-48). Belland does not disclose any method by which those mutations could be identified or characterized. Nothing in Belland teaches or suggests that PCR mutagenesis could be applied to a complete chromosome to generate mutants resistant to a compound or that PCR products of approximately10 kb to approximately 15kb be used. Belland notes that mutations giving rise to ciprofloxacin resistance that occur outside the region analyzed may influence the level of ciprofloxacin resistance but that these mutation would not be detected by the procedures used in Belland (p377, 2nd column, lines 44-48). In contrast with the methods of the present invention the user is able to identify genes that when mutated result in a decreased susceptibility to a compound, even for compounds for which the mechanism of action is unknown. This allows one who is skilled in the art to predict the mechanism of action of any compound with no prior information concerning the molecular target of the compound. Before the disclosure of this invention it had not been shown that a mutation which conferred resistance to a compound could be systematically identified using PCR without prior knowledge of the region with which the compound interacted.

Jones discloses nothing that makes up for the deficiency of Belland. Jones does not discuss mutation of the bacterial chromosome but rather of a plasmid. Jones does not provide any information about how a mutation could be identified within any given stretch of DNA, much less from a complete chromosome.

(16) The Examiner has rejected Claims 22-23 under 35 USC §103(a), as being unpatentable over Belland et al. (Molecular Microbiology, 1994, Vol. 14, No. 2, pp 371-382) in view of Jones et al. (Biotechniques, Vol 10, No. 1, 1991, pages 62-66) and further in view of Wohlstadter et al. (US patent No. 6,087,177). The Examiner cites Wohlstadter as teaching "different methods of mutation exist and may be used in conjunction one with another. Wohlstadter teaches both UV irradiation and chemical mutagens."

Wohlstadter is directed at a rational method for obtaining a novel molecule capable of a desired interaction with a substrate of interest. The disclosure of

Wohlstadter is not related to identification of mutations that confer resistance to a compound. Wohlstadter does nothing to make up for the deficiencies of Belland in combination with Jones. For the reasons given in 15 above, Claim 6 is in compliance with the requirements of 35 USC §103(a), and therefore claims 22-23 which depend from Claim 6 are also in compliance with the requirements of 35 USC §103(a).

For the reasons given above, Applicants believe that all pending claims are in complete compliance with 35 USC §103(a). Applicants respectfully request withdrawal of all Claim rejections under 35 USC §103(a).

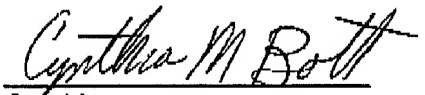
Further, Applicants believe that the response is fully responsive to the prior office action dated August 9, 2002; and therefore, complies with the requirements of 37 CFR 1.121(c) and 37 CFR 1.111.

CONCLUSION

Applicant has properly stated, traversed, accommodated, or rendered moot each of the Examiner's grounds for rejection. Applicant submits that the present application is now in condition for allowance.

If the Examiner has any questions or believes further discussion will aid examination and advance prosecution of the application, a telephone call to the undersigned is invited. If there are any additional fees due in connection with the filing of this amendment, please charge the fees to undersigned's Deposit Account No. 23-0455. If any extensions or fees are not accounted for, such extension is requested and the associated fee should be charged to our deposit account.

Respectfully submitted,



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Dated: December 18, 2003